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Isolation of *Nocardia asiatica* from Cutaneous Ulcers of a Human Immunodeficiency Virus-Infected Patient in Italy▽

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A strain of *Nocardia* was isolated from cutaneous ulcers of a human immunodeficiency virus-infected patient in Italy. Comparative 16S rRNA gene sequence analysis revealed that the isolate represented a strain of *Nocardia asiatica*. Antimicrobial susceptibility testing was essential to guide the clinicians to successfully treat this infection.

CASE REPORT

In July 2005, a human immunodeficiency virus (HIV)-infected 45-year-old male Italian gardener presented with ulcers on the right thigh and a retroauricular ulcer on the left side. On examination, his general condition was found to be poor, and CD4 and CD8 T-cell counts were 189 cells/μl and 1,304 cells/μl, respectively; the patient was treated with antiretroviral drugs lamivudine, didanosine, and efavirenz. Primary cultures from ulcer swabs on blood agar and chocolate agar plates incubated at 37°C in 5% CO_2 yielded small, nonpigmented colonies formed by gram-positive, branched, partially acid-fast, rod-shaped organisms that were considered most likely to represent *Nocardia* spp. Prior to the availability of drug susceptibility results, the patient was empirically treated with trimethoprim-sulfamethoxazole, and after 3 months, a complete resolution of the retroauricular ulcer and a partial remission of the thigh ulcer were observed.

The strain was identified to the genus and species levels by 16S rRNA gene-targeted PCR. Briefly, Mueller-Hinton broth cultures of the strain were centrifuged and resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, 1% Triton X-100 [pH 8]) and incubated at 95°C for 20 min and then on ice for 5 min. Cellular debris was pelleted at 12,000 × *g* for 10 min, and the supernatant containing genomic DNA was used for PCR assay. DNA was amplified with primers NG1 (5′-ACCGACCACAAGGGGG-3′) and NG2 (5′-GGTTGTAAACCTCTTTCGA-3′) for 30 cycles in an iCycler thermal cycler (Bio-Rad, Hercules, CA) under the following conditions: 94°C for 50 s, 55°C for 20 s, and 72°C for 60 s (6). A 596-bp PCR product observed on a 1.2% agarose gel confirmed that the clinical isolate belonged to the *Nocardia* genus. Next, the genomic DNA was used to amplify a 1.5-kb fragment of the 16S rRNA gene, using the universal prokaryotic primers 16S-S (5′-AGAGTTTGATCCTGGCTCAG-3′) and 16S-AS (5′-AGGAGGTGATCCAGCCGCA-3′) (3). For PCR, 3 min at 95°C was followed by 30 cycles of 95°C for 30 s, 55°C for 90 s, and 72°C for 90 s. The PCR products were purified using a QIAquick PCR purification kit (QIAGEN, Hilden, Germany), and automated sequencing was performed at MWG Biotech (Ebersberg, Germany), using the 16S universal primers. BLAST analysis was used to screen sequence databases for strains related to the isolate; the sequence of the PCR product showed a 100% identity with *Nocardia asiatica*. The results of the biochemical tests performed as previously described (4–5, 8) were also consistent with those determined for the species *N. asiatica*: a positive reaction for acid production from glucose and rhamnose and for citrate utilization and a negative reaction for acid production from sorbitol, arabinose, and erythritol.

N. asiatica was reported to be susceptible to the drugs imipenem and tobramycin (5, 8), but no major information is known for other drugs (10). Antibiotic susceptibility profiles differ among species of *Nocardia* (2, 9); thus the determination of MICs for rarely encountered species is necessary for correct therapeutic management. MICs of the *N. asiatica* isolate were determined using a standard twofold dilution of antimicrobials in Mueller-Hinton broth according to the National Committee for Clinical Laboratory Standards (NCCLS; now Clinical and Laboratory Standards Institute) (7), but instead of the microdilution method in microtiters, tests were performed in tubes containing 1-ml volumes. The organism was shown to be susceptible to amikacin, tobramycin, gentamicin, imipenem, ceftriaxone, cefotaxime, linezolid, doxycycline (MICs of ≤0.25, 2, 1, 0.5, 2, 2, 2, and 1 μg/ml, respectively) and resistant to ciprofloxacin and trimethoprim-sulfamethoxazole (MICs of >16 and 4/76 μg/ml, respectively). Intermediate susceptibility was shown for clarithromycin (MICs of 4 μg/ml). The MICs of moxifloxacin and levofloxacin were both >8 μg/ml, but no assignment of resistance or susceptibility could be made because no breakpoints are indicated for these drugs in the NCCLS protocols.

In January 2006, *N. asiatica* was isolated again from the thigh ulcer of the patient. On the basis of antimicrobial sus-

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ceptibility testing, the patient was treated with doxycycline for 5 months; following this therapy, a full clinical recovery was observed.

Nocardioses are infections caused by soilborne aerobic microorganisms belonging to the genus *Nocardia*. Eighty species of *Nocardia* have been described (http://www.bacterio.cict.fr/n/nocardia.html), and several are recognized as human and/or animal pathogens, causing diseases ranging from pulmonary or central nervous system infections in immunocompromised patients to cutaneous infections in normal hosts (2). Traditional differentiation of species based on biochemical characterization is laborious and time-consuming and has been in part replaced by more rapid molecular methods.

In 2004, a new species of *Nocardia*, *N. asiatica*, was described, with five strains isolated in Asia over the period of 1985 to 1999 from patients with nocardiosis in Japan and from clinical specimens from Thailand; three strains were isolated from sputum, one from a granuloma, and one from transtracheal aspirate (5).

This is the first report of the isolation of *N. asiatica* outside of Asia. *Nocardiae* are ubiquitous in the environment and are considered saprophytic soil microorganisms primarily responsible for the decomposition of organic plant material (1). The patient infected with *N. asiatica* reported here is a gardener, so it is possible that aerosolization and dispersal of grass and soil closely related to his job may have facilitated skin infection. Nocardial diseases are rare in humans, occurring most frequently in immunocompromised patients; organisms are usually acquired by inhalation, and rarely, direct skin inoculation is implicated (1). The observation that *N. asiatica* was isolated from a cutaneous ulcer of an HIV-infected patient is in keeping with this knowledge.

Overall, our observations indicated that accurate species identification and antimicrobial susceptibility testing of *N. asiatica* are essential for successful treatment of this infection.

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